UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

PFIZER INC, *et al.*,	)	
	)	
Plaintiffs/Counter-Defendants,	)	
	)	
v.	)	Civil Action No.: 08 CV 02018 LAK
	)	
MATHEW I. GELFAND, M.D.,	)	
	)	
Defendant/Counter-Plaintiff.	)	
	)	

DEFENDANT/COUNTER-PLAINTIFF'S COUNTERCLAIMS

JURY TRIAL DEMANDED

Defendant and Counter-Plaintiff Mathew I. Gelfand, M.D., ("Dr. Gelfand"), by and through his undersigned counsel, hereby files his counterclaims against Plaintiffs and Counter-Defendants Pfizer Inc. ("Pfizer"), Robert Jarvik ("Jarvik"), and Jarvik Heart, Inc. ("JHI") (together, "Counter-Defendants"), and states as follows:

1. This is an action by Dr. Gelfand against Pfizer, Jarvik, and JHI for infringement of United States Patent No. 5,837,688 (hereinafter, the "'688 Patent").

PARTIES, VENUE, AND JURISDICTION

2. Dr. Gelfand is a practicing physician, licensed by the State of New York, with a specialty in internal medicine, hematology, and blood circulation. Dr. Gelfand is a citizen of New York, with an address at 245 Fairway Road, Lido Beach, New York, New York 11561.

3. Pfizer is a corporation organized and existing under the laws of the State of Delaware with a principal place of business at 235 East 42nd Street, New York, New York 10017.

4. Jarvik is world-renown medical engineer, is well-known among physicians who specialize in treating cardiovascular disease. Jarvik resides in New York, New York, and serves as President and Chief Executive Officer of JHI. Jarvik has never been, and is not now, licensed to practice medicine in any state and he is not a cardiologist.

5. JHI is a New York corporation located at 333 West 52nd Street, New York, New York 10019, in business to promote treatment of cardiovascular disease, including coronary heart disease, in humans.

6. This action arises under the patent laws of the United States, Title 35, United States Code. This Court has subject matter jurisdiction over this action pursuant to 28 U.S.C. §§ 1331 and 1338(a).

7. Venue is proper in this District as to all Counter-Defendants pursuant to 28 U.S.C. § 1400(b) and, as to Counter-Defendants Pfizer and JHI, pursuant to 28 U.S.C. § 1391(b).

CONDITIONS PRECEDENT

8. Any and all conditions precedent for bringing or maintaining this cause of action have been satisfied by Dr. Gelfand, or are waived or excused by Counter-Defendants, and each of them.

FACTS

9. On application Serial Number 758,615, filed by Dr. Gelfand on November 27, 1996, the United States Patent and Trademark Office issued the ’688 Patent, entitled “Use of Thrombolytic Reagent for Prevention of Vascular Disease,” on November 17,

1998. A copy of the '688 Patent is attached hereto as Exhibit A and incorporated herein as if stated in full.

10. Pursuant to the '688 Patent, Dr. Gelfand owns and controls the right to preclude others, including Pfizer, Jarvik, and JHI, from practicing, from selling, and from inducing the sale of a process by which a thrombolytic reagent with fibrinolytic activity is chronically administered to humans in low doses over long periods of time to treat vascular disease, including cardiovascular disease and cerebral vascular disease, *e.g.*, coronary heart disease, myocardial infarction or heart attack, and stroke.

11. The '688 Patent defines such thrombolytic reagents as drugs that reduce blood clots and, therefore, induce angiogenesis, *i.e.*, "drugs that act on the endogenous fibrinolytic system by converting plasminogen to the potent proteolytic enzyme plasmin. Plasmin in turn degrades fibrin clots and other plasma proteins."

12. The thrombolytic reagents that can be used in the practice of the '688 Patent includes "thrombolytic reagents such as tissue plasminogen activator" (t-PA) and, more broadly, all "delivery systems that provide for long-term sustained release of thrombolytic reagents, such as t-PA, in the blood, which is effective as a means for preventing the development of vascular disease."

13. As stated in the '688 Patent:

> The object of the invention is the prevention or dissolving of clots as they form in the vascular system of the treated patient. In accordance with the present invention, the object can be achieved through the use of t-PA preparations designed for sustained release of t-PA into the bloodstream of a patient over prolonged periods of time.

14. At the time that the '688 Patent became effective, it was generally accepted in the field of medicine: (a) that the human fibrinolytic system basically consists

of a balance between clotting factors, such plasminogen activator inhibitor type 1 ("PAI-1"), and anti-clotting factors, such as t-PA; and (b) that an increase in t-PA activity in the blood and/or endothelial cells results in a decrease of PAI-1 activity therein as well.

15. On or about December 18, 1996, the United States Food and Drug Administration ("FDA") approved Pfizer's application for the sale of a statin product, the calcium salt of atorvastatin or "atorvastatin calcium," which compound Pfizer thereafter has sold and sells, and offered and offers for sale, in interstate commerce as Lipitor®. In 2002, FDA approved Pfizer to market doses of Lipitor® at doses as low as 10mg per day.

16. On September 30, 2003, Pfizer submitted to FDA a Supplemental New Drug Application ("SNDA") for Lipitor® "based on the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) lipid lowering arm results."

17. Dr. Gelfand first received the SNDA on January 25, 2008, after a three-year delay from FDA on his Freedom of Information Act request of September 26, 2005.

18. In its September 2003 SNDA for Lipitor®, Pfizer stated that the ASCOT lipid-lowering test results "support[] a new indication for the prevention of cardiovascular disease in patients without clinically evident coronary heart disease."

19. Pfizer further stated that the ASCOT lipid-lowering test results showed that Lipitor® at 10mg tablets conferred "additional protection" against coronary heart disease. Pfizer wrote to FDA:

> On September 2, 2002, the Data Safety Monitoring Board (DSMB) of ASCOT proposed to the Steering Committee that the double-blind lipid-lowering arm of ASCOT be terminated due to a highly significant reduction in the primary endpoint of coronary heart disease and a significant reduction in stroke incidence in those patients receiving Lipitor compared to a placebo. The magnitude of the benefit exceeded the predefined stopping rule for this part of the trial. The ASCOT Steering

> Committee accepted this recommendation on October 4, 2002 and a decision to close this section of the study was taken.

20. Since at least 2003, physicians treating patients for risks associated with cardiovascular and/or cerebral vascular disease, including coronary heart disease and stroke, have known that blood clotting is the major risk factor for the occurrence of heart attack and stroke and that vascular angiogenesis is the major risk prevention factor for the occurrence of heart attack and stroke.

21. Since at least 2003, medical literature published in the United States has explored and extolled the benefits of statin compounds, including Lipitor®, for their benefit as a chronically administered or sustained-release thrombolytic and fibrinolytic reagent.

22. The thrombolytic and fibrinolytic properties of Lipitor® are the only medically reasonable explanation for the statements that Pfizer made to FDA in Pfizer's September 2003 SNDA to FDA about the effects of Lipitor® beyond cholesterol-lowering.

23. At some time after September 2003, Pfizer began a highly successful national marketing campaign for the sale of Lipitor® for its effect as a chronically administered or sustained-release thrombolytic and fibrinolytic reagent. In 2006 alone, Americans filled more than 79 million prescriptions for Lipitor®, accounting for roughly $14 billion in domestic sales of Lipitor®.

24. As early as September 2003, and as a material part of its national campaign to promote Lipitor®, Pfizer actively induced the infringement of the '688 Patent by inducing physicians in the United States to prescribe Lipitor®, and patients to use Lipitor®, for its effects beyond cholesterol-lowering, *i.e.*, as a chronically

administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

25. In 2004, Pfizer obtained approval from FDA and began to manufacture, sell, and offer for sale a compound drug with trade name Caduet®, a drug that combines Pfizer's atorvastatin calcium (Lipitor®) with Pfizer's amlodipine product (Norvasc®) to treat cardiovascular disease.

26. In 2004, the American Journal of Hypertension ("AJH") reported:

> Data from the recent Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) support the view that statins protect hypertensive patients from end-organ damage, not only through cholesterol reduction but also through other pathways. These include a direct modulation of the endothelial function, as well as an interaction with the fibrinolytic activity. In this regard, evidence from in vitro studies indicate [*sic*] that statins positively affect the fibrinolytic system of cultured smooth muscle cells as well as endothelial cells.

(footnotes omitted). The AJH report is attached hereto as Exhibit B.

27. The AJH report concluded in material part:

(a) that "amlodipine monotherapy . . . significantly increased t-PA activity" in the human vascular system;

(b) that "atorvastatin monotherapy (ie, a significant decrease in PAI-1 activity and an increase in t-PA activity) confirm the findings of some in vitro and in vivo studies"; and

(c) that "the combination of amlodipine and atorvastatin improved the fibrinolytic balance more than the single monotherapy."

28. On August 5, 2005, Dr. Gelfand put Pfizer on notice that its manufacture, use, and sale of Lipitor® infringes on the '688 Patent. In his letter, Dr. Gelfand offered Pfizer the opportunity to license the '688 Patent.

29. On September 14, 2005, Pfizer responded to Dr. Gelfand's notice as follows:

> Lipitor has no indications that are dependent upon anti-thrombotic or fibrinolytic activity and there is no plan to pursue such an indication. The Pfizer team therefore concluded that [the '688 Patent] would have minimal value to Pfizer and there was no interest in further discussing this licensing opportunity.

The September 14, 2005, Letter from Ann C. Barry, Ph.D., Pfizer's Director of Licensing & Development, is attached hereto as Exhibit C ("Barry Letter"), and is incorporated herein as if stated in full.

30. Through the Barry Letter, Pfizer intentionally misrepresented its promotion of Lipitor®, its September 2003 SNDA to FDA for Lipitor®, and its plans for promoting Lipitor® and Caduet®.

31. Dr. Gelfand relied on Pfizer's misrepresentations to his detriment by not filing this suit prior to his receipt in January 2008 of Pfizer's September 2003 SNDA for Lipitor®. The SNDA reveals Pfizer's lie in the Barry Letter, Pfizer's bad faith towards Dr. Gelfand from as early September 2005 (if not before), and Plaintiffs/Counter-Defendants' willful infringement of the '688 Patent since September 2005.

32. On April 13, 2006, Jarvik entered into a two-year contract by which Jarvik is to receive $550,000 in the first year and $800,000 in the second year in return for his promotion of Lipitor®, including for its effects beyond cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

33. On information and belief, Jarvik has all or nearly all sums due under the initial two-year contract with Pfizer.

34. In his contract with Pfizer, Jarvik reserved control of his association with Pfizer's promotion of Lipitor®. Jarvik reserved "the right, in [his] discretion, to refuse to have a statement attributed to [Jarvik] if [he] believes in good faith that such statement is untrue."

35. Jarvik has exercised that control. Jarvik and JHI have stated publicly:

> I have the training, experience, and medical knowledge to understand the conclusions of the extensive clinical trials that have been conducted to study the safety and effectiveness of Lipitor. Also, Pfizer submits advertising concepts in advance to the FDA for review and comment. The statements included in the ads fairly represent the scientific truth about Lipitor, which the public has a right to know, and which Pfizer is entitled to teach.
>
> I accepted the role of spokesman for Lipitor because I am dedicated to the battle against heart disease . . . . I believe the process of educating the public is beneficial to many patients and I am pleased to be part of an effort to reach them.
>
> I am not a celebrity. I am a medical scientist specializing in advanced technology to treat heart failure who understands that no one in his or her right mind would want an artificial heart if it could be avoided with preventive medicine.

36. For its part in its contract with Jarvik, Pfizer reserved the right to promote Lipitor® in ways that satisfies Jarvik: "In the event [Pfizer], in its sole discretion, requests approval from [Jarvik] with respect to any materials or any particular element, [Jarvik] shall provide comments, if any, in writing, within twenty-four (24) hours of [Jarvik's] receipt of such materials or element. In connection with the preceding sentence, [Jarvik] shall have the right to review all press releases prior to the general commercial distribution of such releases."

37. Pfizer prepared, and Jarvik approved, either tacitly or in writing, those advertisement that feature Jarvik's endorsement and promotion of Lipitor® for its effects

beyond cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

38. The benefits that Jarvik has received by his promotion of Lipitor® for its effects beyond cholesterol-lowering *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke, include heightened medical, media, and financial attention for JHI.

39. Since not later than April 2006, Pfizer and/or Jarvik have, directly and indirectly, urged physicians and their patients at risk for heart attack and/or stroke to use Lipitor® for its effects beyond cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke. Such inducements include, without limitation:

(a) On October 15, 2006, Pfizer's President's J. Patrick Kelly was quoted in The New York Times: "By taking any dose of Lipitor, you will reduce the risk of a cardiovascular event faster and to a greater degree than you will with any other medicine."

(b) On September 7, 2006, the Boston Globe reported that Pfizer has sent thousands of sale representatives to convince physicians that Lipitor® is more effective in the prevention of heart disease than any other or generic statin.

(c) Beginning in 2006, Counter-Defendants began to run advertisement LP27879-I, and others like it, in major national newspapers. The newspaper advertisements features Jarvik, who extols Lipitor® for its effects beyond cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

(d) Beginning in 2006, Counter-Defendants began to run television and internet or web advertisements for Lipitor®. These advertisements feature Jarvik extolling Lipitor® for its effects beyond cholesterol-lowering, *i.e.,* as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

40. From time to time since September 2003, Pfizer has directly and indirectly infringed on the ’688 Patent by offering for sale, selling, and inducing doctors and their patients to use Lipitor® and Caduet® as a chronically administered thrombolytic reagent for the prevention of vascular disease, including without limitation as a chronically administered reagent for reducing or otherwise affecting blood clotting or the fibrinolytic system in patients at risk for heart disease and/or stroke.

41. From time to time since April 13, 2006, Jarvik, for his own benefit and for the benefit of JHI, has directly and indirectly infringed on the ’688 Patent by offering for sale, selling, and inducing doctors and their patients to use Lipitor® as a chronically administered thrombolytic reagent for the prevention of vascular disease, including without limitation as a chronically administered reagent for reducing or otherwise affecting blood clotting or the fibrinolytic system in patients at risk for heart disease and/or stroke.

42. From time to time since April 13, 2006, JHI has, through Jarvik, directly and indirectly infringed on the ’688 Patent by offering for sale, selling, and inducing doctors and their patients to use Lipitor® as a chronically administered thrombolytic reagent for the prevention of vascular disease, including without limitation as a

chronically administered reagent for reducing or otherwise affecting blood clotting or the fibrinolytic system in patients at risk for heart disease and/or stroke.

43. On February 25, 2008, Dr. Gelfand made an attempt to resolve this dispute with Counter-Defendants by sending a written request for a meeting to Jarvik and to Allen Waxman, the General Counsel of Pfizer.

44. In response to Dr. Gelfand's request for a meeting, Counter-Defendants filed the complaint for declaratory judgment that commenced this action.

FIRST CLAIM FOR RELIEF:
COUNTER-DEFENDANTS' INFRINGEMENT OF THE '688 PATENT
IN VIOLATION OF 35 U.S.C. 271(a)

45. Dr. Gelfand realleges paragraphs 1 through 44 above as if fully set forth herein.

46. The '688 Patent is valid and enforceable, and has been since at least 2003 and throughout the period from April 13, 2006, to date.

47. In violation of 35 U.S.C. §271(a), Counter-Defendants, and each of them, have infringed and violated the '688 Patent by selling and offering to sell Lipitor® within the United States -- without authority of Dr. Gelfand -- for Lipitor®'s effects beyond cholesterol-lowering, *i.e.,* as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

48. In violation of 35 U.S.C. §271(a), Counter-Defendants, and each of them, have infringed and violated the '688 Patent by selling and offering to sell Caduet® within the United States -- without authority of Dr. Gelfand -- for Caduet®'s effects beyond cholesterol-lowering, *i.e.,* as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

49. Pfizer infringed the ’688 Patent in bad faith and willfully, including through Pfizer’s misrepresentations in the Barry Letter of September 2003.

50. As a result of Counter-Defendants’ infringement of the ’688 Patent, Dr. Gelfand has suffered substantial damages within the meaning of 35 U.S.C. §284.

51. Dr. Gelfand will be irreparably harmed if Counter-Defendants are not enjoined from infringing the ’688 Patent.

SECOND CLAIM FOR RELIEF:
COUNTER-DEFENDANTS’ ACTIVE INDUCING INFRINGEMENT OF THE ’688 PATENT
IN VIOLATION OF 35 U.S.C. 271(b)

52. Dr. Gelfand realleges paragraphs 1 through 44 above as if fully set forth herein.

53. The ’688 Patent is valid and enforceable, and has been since at least 2003 and throughout the period April 13, 2006, to date.

54. In violation of 35 U.S.C. §271(b), Counter-Defendants, and each of them, have actively induced doctors to infringe the ’688 Patent by inducing such doctors to use, prescribe, and otherwise require their patients to purchase Lipitor® within the United States -- without authority of Dr. Gelfand – to secure Lipitor®’s effects beyond cholesterol-lowering, *i.e.,* as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

55. In violation of 35 U.S.C. §271(b), Counter-Defendants, and each of them, have actively induced doctors to infringe the ’688 Patent by inducing such doctors to use, prescribe, and otherwise require their patients to purchase Caduet® within the United States -- without authority of Dr. Gelfand – to secure Caduet®’s effects beyond

cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

56. Pfizer infringed the ’688 Patent in bad faith and willfully, including through Pfizer’s misrepresentations in the Barry Letter of September 2003.

57 As a result of Counter-Defendants’ infringement of the ’688 Patent, Dr. Gelfand has suffered substantial damages within the meaning of 35 U.S.C. §284.

58. Dr. Gelfand will be irreparably harmed if Counter-Defendants are not enjoined from infringing Dr. the ’688 Patent.

THIRD CLAIM FOR RELIEF:
COUNTER-DEFENDANT PFIZER’S INFRINGEMENT OF THE ’688 PATENT IN VIOLATION OF 35 U.S.C. 271(e)(2)(A)

59. Dr. Gelfand realleges paragraphs 1 through 44 above as if fully set forth herein.

60. The ’688 Patent is valid and enforceable, and has been since at least 2003 and throughout the period April 13, 2006, to date.

61. Pfizer’s SNDA of September 2003 sought authority from FDA to promote Lipitor® for its effects beyond cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

62. Pfizer’s NDA of 2004 sought authority from FDA to promote Caduet® for its effects beyond cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

63. Pfizer's SNDA of September 2003 constitutes an "application . . . described by section 505(b)(2)" of the Federal Food, Drug, and Cosmetic Act "for a drug claimed" in the '688 Patent or "the use of which is claimed" in the '688 Patent, all within the meaning of 35 U.S.C. §271(e)(2)(A).

64. Pfizer's NDA of 2004 constitutes an "application . . . described by section 505(b)(2)" of the Federal Food, Drug, and Cosmetic Act "for a drug claimed" in the '688 Patent or "the use of which is claimed" in the '688 Patent, all within the meaning of 35 U.S.C. §271(e)(2)(A).

65. In submitting its SNDA of September 2003 to FDA, Pfizer infringed the '688 Patent in violation of 35 U.S.C. §271(e)(2)(A).

66. In submitting its NDA of 2004 to FDA, Pfizer infringed the '688 Patent in violation of 35 U.S.C. §271(e)(2)(A).

67. Counter-Defendant Pfizer infringed the '688 Patent in bad faith and willfully, including through Pfizer's misrepresentations in the Barry Letter of September 2003.

68. As a result of Plaintiffs/Counter-Defendants' infringement of the '688 Patent, Dr. Gelfand has suffered substantial damages within the meaning of 35 U.S.C. §284.

69. Dr. Gelfand will be irreparably harmed if Plaintiffs/Counter-Defendants are not enjoined from infringing the '688 Patent.

FOURTH CLAIM FOR RELIEF:
COUNTER-DEFENDANT PFIZER'S INFRINGEMENT OF THE '688 PATENT IN VIOLATION OF 35 U.S.C. 271(a)

70. Dr. Gelfand realleges paragraphs 1 through 44 above as if fully set forth herein.

71. The '688 Patent is valid and enforceable, and has been since at least 2003 and throughout the period from April 13, 2006, to date.

72. In violation of 35 U.S.C. §271(a), Pfizer has infringed and violated the '688 Patent by making Lipitor® within the United States -- without authority of Dr. Gelfand -- for Lipitor®'s effects beyond cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

73. In violation of 35 U.S.C. §271(a), Pfizer has infringed and violated the '688 Patent by making Caduet® within the United States -- without authority of Dr. Gelfand -- for Caduet®'s effects beyond cholesterol-lowering, *i.e.*, as a chronically administered thrombolytic reagent in the treatment or prevention of cardiovascular disease, including heart attack and stroke.

74. Pfizer infringed the '688 in bad faith and willfully, including through Pfizer's misrepresentations in the Barry Letter of September 2003.

75. As a result of Pfizer's infringement of the '688, Dr. Gelfand has suffered substantial damages within the meaning of 35 U.S.C. §284.

76. Dr. Gelfand will be irreparably harmed if Pfizer is not enjoined from infringing the '688 Patent.

WHEREFORE, Dr. Gelfand requests the following relief from Counter-Defendants, and each of them, as follows:

A. a judgment of the Court for damages against Counter-Defendants, jointly and severally, adequate to compensate Dr. Gelfand for Counter-Defendants' infringement of the '688 Patent, but not less than one percent (1%) of the net sales of Lipitor® for each year commencing April 13, 2006 through the expiration of the '688 Patent plus 30 months (to compensate for time lost to Dr. Gelfand by Pfizer's misrepresentations in the Barry Letter), all as allowed by 35 U.S.C. §284;

B. a judgment of the Court for damages against Counter-Defendants, jointly and severally, adequate to compensate Dr. Gelfand for Counter-Defendants' infringement of the '688 Patent, but not less than one percent (1%) of the net sales of Caduet® for each year commencing April 13, 2006 through the expiration of the '688 Patent plus 30 months (to compensate for time lost to Dr. Gelfand by Pfizer's misrepresentations in the Barry Letter), all as allowed by 35 U.S.C. §284;

C. an order of this Court preliminarily and then permanently enjoining Pfizer from making Lipitor® and/or Caduet® until the expiration of the '688 Patent plus 30 months (to compensate for time lost to Dr. Gelfand by Pfizer's misrepresentations in the Barry Letter), all as allowed by 35 U.S.C. §283;.

D. an order of this Court preliminarily and then permanently enjoining Counter-Defendants from selling, offering for sale, or inducing the use of Lipitor® and/or Caduet® until the expiration of the '688 Patent plus 30 months (to compensate for time lost to Dr. Gelfand by Pfizer's misrepresentations in the Barry Letter), all as allowed by 35 U.S.C. §283;.

E. Treble damages as allowed by 35 U.S.C. §284;

F. Attorneys' fees in this action pursuant to 35 U.S.C. § 285;

G. Costs and expenses in this action; and

H. Such further and other relief as this Court may deem just and proper.

JURY TRIAL DEMAND

Dr. Gelfand hereby demands trial by jury as to all issues triable as of right by jury.

Dated: March 24, 2008
Bethesda, Maryland

Respectfully Submitted,

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CERTIFICATE OF SERVICE

I HEREBY CERTIFY that, on this 24th day of March, 2008, I caused a copy of the foregoing **DEFENDANT/COUNTER-PLAINTIFF MATHEW I. GELFAND'S, M. D., COUNTERCLAIMS** to be delivered via ECF filing and by United States Mail, postage prepaid, to:

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Exhibit A

US005837688A

United States Patent [19]

Gelfand

[11] Patent Number: 5,837,688

[45] Date of Patent: Nov. 17, 1998

[54] **USE OF THROMBOLYTIC REAGENTS FOR PREVENTION OF VASCULAR DISEASE**

[76] Inventor: **Mathew I. Gelfand**, 245 Fairway Rd., Lido Beach, N.Y. 11561

[21] Appl. No.: **758,615**

[22] Filed: **Nov. 27, 1996**

[51] **Int. Cl.**[6] **A61K 38/00**

[52] **U.S. Cl.** **514/21**; 514/2

[58] **Field of Search** 514/2, 21

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,853,330	8/1989	Goeddel et al.	435/226
5,156,969	10/1992	Gill et al.	435/240.2
5,262,170	11/1993	Anderson et al.	424/94.64
5,288,503	2/1994	Wood et al.	424/497
5,385,732	1/1995	Anderson et al.	424/94.64
5,426,097	6/1995	Stern et al.	514/12

FOREIGN PATENT DOCUMENTS

297860 B1	4/1989	European Pat. Off. .
0 199 574	10/1991	European Pat. Off. .
0 297 860	9/1993	European Pat. Off. .

OTHER PUBLICATIONS

Bick et al., "Thrombolytic Therapy and Its Uses", Lab .Med. 26:330–337, May 1995.

Shabahang et al., 1994, "The Clinical Impact of Risk Factor and Prophylaxis on Pulmonary Embolism", J. Vasc. Dis. 45:749–754.

Vipond et al., 1994, "Experimental Adhesion Prophylaxis with Recombinant Tissue Ploasminogen Activator", Ann. R. Coll. Surg. Engl. 76:412–415.

Mohr et al., 1988, "Recent Advances in the Management of Venous Thromboembolism", Mayo Clin. Proc. 63:281–290.

Pannekoek et al., 1988, "Mutants of Human Tissue–Type Plasminogen Activator (t–PA): Structural Aspects and Functional Properties", Fibrinolysis 2:123–132.

Rose et al., 1988, "Plasminogen Activators", Ann. Rep. Med. Chem. 23:111–119.

Harris, 1987, "Second–Generation Plasminogen Activators", Prot. Eng. 1:449–458.

Fass and Toole, 1985, "Genetic Engineering and Coagulation Factors", Clin. Haem. 14:547–570.

Primary Examiner—Marianne M. Cintins
Assistant Examiner—Dwayne C. Jones
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[57] **ABSTRACT**

The present invention relates to the administration of thrombolytic reagents such as tissue plasminogen activator (t-PA), streptokinase and/or urokinase, over prolonged periods of time for prevention of vascular disease such as cerebral vascular thrombosis, pulmonary embolism, deep venous thrombus, acute myocardial infarction and fresh or aged arterial thrombi. The invention relates generally to delivery systems that provide for sustained release of thrombolytic reagents such as tissue plasminogen activator (t-PA), streptokinase and/or urokinase, over prolonged periods of time. The thrombolytic reagents may be administered, for example, transdermally, topically, intranasally or orally.

17 Claims, No Drawings

USE OF THROMBOLYTIC REAGENTS FOR PREVENTION OF VASCULAR DISEASE

INTRODUCTION

The present invention relates to the administration of thrombolytic reagents such as tissue plasminogen activator (t-PA), streptokinase and/or urokinase, over prolonged periods of time for prevention of vascular disease such as cerebral vascular thrombosis, pulmonary embolism, deep venous thrombus, acute myocardial infarction and fresh or aged arterial thrombi. The invention relates generally to delivery systems that provide for sustained release of thrombolytic reagents such as tissue plasminogen activator (t-PA), streptokinase and/or urokinase, over prolonged periods of time. The thrombolytic reagents may be administered, for example, transdermally, topically, intranasally or orally.

BACKGROUND OF THE INVENTION

TISSUE PLASMINOGEN ACTIVATOR

Thrombolytic drugs act on the endogenous fibrinolytic system by converting plasminogen to the potent proteolytic enzyme plasmin. Plasmin in turn degrades fibrin clots and other plasma proteins. A number of thrombolytic drugs, including urokinase, streptokinase and t-PA, are currently used to treat acute vascular disease.

Tissue plasminogen activator (t-PA) activates plasminogen to generate the proteinase plasmin which plays an important role in the degradation of fibrin. t-PA has been a particularly important pharmaceutical agent for use in treatment of vascular diseases due to its ability to dissolve blood clots in vivo. t-PA was originally identified and purified from natural sources. Through the use of recombinant DNA techniques, DNA clones encoding the t-PA molecule have recently been identified and characterized leading to a determination of the DNA sequence and deduced amino acid sequence of t-PA (U.S. Pat. No. 4,853,330).

Several variants of t-PA have also been developed that address some of the disadvantages associated with the use of t-PA. These disadvantages include the short half life and fast clearance rate of t-PA. Such variants include those described in EPO Patent Publication No. 199,574, that have amino acid substitutions at the proteolytic cleavage sites at amino acid positions 275, 276 and 277. These forms are referred to as protease-resistant one-chain t-PA variants in that, unlike natural t-PA, they exist in either one chain or two chain form, are resistant to proteolytic cleavage and exist in one-chain form. Such variants are thought to be superior to natural t-PA for pharmaceutical uses in that they are more stable. In addition, a variety of glycosylation mutants exist at positions 117, 119, 184–186 and 448–450 which exhibit higher specific activity than natural t-PA.

A general review of plasminogen activators and derivatives thereof can be found in Harris (1987, Protein Engineering 1:449–458); Pannekock et al. (1988, Fibrinolysis 2:123–132); and Ross et al. (1988, Annual Reports in Medicinal Chemistry, Vol. 23, Chapter 12), each of which is incorporated by reference herein.

VASCULAR DISEASE

Thrombosis and its complications are considered the most frequent causes of morbidity and death in the adult population. Pulmonary embolism is estimated to be the third most common cause of death in the United States (Mohr et al., 1988, Mayo Clin. Proc. 63:281–290). At present, anticoagulation is the basic approach to treatment of thromboembolic disorders (Bick, R. et al., 1995, Laboratory Medicine 26:330–337; Shabahang, M. et al., 1994, Angiology 45:749–754). Pharmaceutical preparations containing thrombolytic reagents such as t-PA, urokinase and streptokinase are currently used for treatment of acute vascular disease.

Short term administration of pharmaceutical preparations containing thrombolytic reagents, such as t-PA, urokinase or streptokinase, are currently used to treat patients suffering from cardiovascular diseases or conditions. For example, t-PA is parentally administered to patients as a means for treatment of deep vein thrombosis or peripheral vascular disease. t-PA is also used in connection with emergency medical care facilities for treatment of arterial embolisms which include pulmonary and extremity embolisms and infarction.

The deposition of fibrin in the peritoneal cavity may lead to fibrous adhesion formation which are the most common cause of small bowel obstruction in developed countries (Vipond et al., 1994, Ann. R. Coll. Surg. Engl. 76:412–415; EP 0297860 B1). t-PA has also been used successfully to prevent fibrin deposition or adhesion formation in the peritoneal cavity following surgery, infection, trauma or inflammation.

SUMMARY OF THE INVENTION

The present invention relates to methods for preventing vascular disease by the chronic administration of low doses of thrombolytic reagents such as tissue plasminogen activator (t-PA), streptokinase and/or urokinase, over prolonged periods of time. The present invention also relates to delivery systems that can be used in the methods of the invention. For example, systems that provide for sustained release of thrombolytic reagents, such as t-PA, over prolonged periods of time can be used. In general, the total daily dose range of t-PA should be sufficient to achieve serum concentrations of between about 1 and 50 mgs. For example, between about 1 and 50 mgs of a daily parenteral dose may be administered, most preferably a daily dose range should be between 10 and 30 mgs of t-PA. Therefore, an object of the invention is to provide dose-controlling applicators for thrombolytic compositions such as t-PA.

The present invention may be used therapeutically as a prophylactic means for inhibiting the development of vascular diseases such as pulmonary embolus, deep venous thrombus, acute myocardial infarction and fresh or aged arterial thrombi. The invention is of particular use for treatment of individuals at high risk for vascular disease, such as, diabetics, hypertensive or hyperlipidemia patients, smokers or those individuals with a family history of vascular disease.

The present invention encompasses a number of preferred embodiments. In the first, the thrombolytic reagent is contained in a dermal patch which may be used to provide sustained release of tissue plasminogen activator into a patient's bloodstream over prolonged periods of time. In another embodiment of the invention the thrombolytic reagent may be combined with slow release gel formulations which may be applied topically to the patient. In yet another embodiment of the invention the thrombolytic reagent may be added to a biocompatible matrix material which may be implanted into the body of the patient for slow sustained release of the reagent. The thrombolytic reagent may also be administered orally or intranasally through the use of nasal sprays containing the reagent.

DETAILED DESCRIPTION OF THE INVENTION

Thrombosis and its complications are considered the most frequent causes of morbidity and death in the adult popu-

lation. The present invention involves a prophylactic method for inhibiting the development of vascular disease such as pulmonary embolus, deep venous thrombus and acute myocardial infarction and cerebral vascular thrombus. The invention relates to the chronic administration of low doses of thrombolytic reagents to prevent vascular disease. The thrombolytic reagents may be administered daily, weekly, monthly or yearly depending on the type of delivery system utilized. The desired goal of any such delivery systems is a constant long term delivery of thrombolytic reagents. Such thrombolytic reagents include, for example, t-PA, streptokinase and urokinase, etc.

The invention is of particular use for treatment of individuals at high risk for vascular disease, such as, diabetics, hypertensive or hyperlipidemia patients, smokers or those individuals with a family history of vascular disease. In such patients, the delivery of a continuous sustained release of thrombolytic reagents, such as t-PA, streptokinase or urokinase, may prevent the development of vascular disease.

Thus, the present invention relates to the chronic administration of low doses of thrombolytic reagents such as tissue plasminogen activator, streptokinase and/or urokinase over prolonged periods of time for prevention of vascular disease. The invention further relates to delivery systems that provide for long-term sustained release of thrombolytic reagents, such as t-PA, in the blood, which is effective as a means for preventing the development of vascular disease. The object of the invention is the prevention or dissolving of clots as they form in the vascular system of the treated patient. In accordance with the present invention, the object can be achieved through the use of t-PA preparations designed for sustained release of t-PA into the bloodstream of a patient over prolonged periods of time.

THROMBOLYTIC REAGENTS

The thrombolytic reagents to be used in the practice of the invention, herein defined as any reagents which have fibrinolytic activity, may be derived from a variety of different sources. For example, the t-PA may be produced in large quantities using recombinant DNA techniques well known to those skilled in the art such as those disclosed in U.S. Pat. No. 4,853,330 which is incorporated herein by reference. Alternatively, the t-PA may be obtained from a number of commercially available sources such as Activase© supplied by Genentech, Inc.

When using t-PA, it is within the scope of the invention that variants of naturally occurring t-PA may also be used in the practice of the invention. In preferred embodiments, such variants of t-PA may have an increased half life or a slower rate of clearance from the body. For example, variants having amino acid substitutions at the proteolytic cleavage sites at position 275, 276 and 277 which render t-PA preparations more stable may be used. Glycosylation mutants at amino acids 117–119, 184–186 and 448–45 exhibit a higher specific activity and such variant may also be used in the practice of the invention. t-PA can also be modified to delete amino acids 51–87 which results in a variant having a slower clearance from plasma. These variants represent only a subset of the known variants of t-PA which may be used in the presently claimed delivery systems.

It is also within the scope of the present invention that thrombolytic reagents other than t-PA may be used in the practice of the invention. Such agents include urokinase and streptokinase both of which may be obtained from commercial sources (Urokinase, Abbott Laboratories; Streptokinase, Pharmacia Adria).

METHOD OF PREVENTING VASCULAR DISEASE

The present invention relates to methods of preventing vascular disease by chronic administration of low doses of thrombolytic reagents. The present invention may be used as a prophylactic means for inhibiting the development of vascular diseases such as cerebral vascular thrombosis, pulmonary embolus, deep venus thrombus and acute myocardial infarction. The invention is of particular use for treatment of individuals at high risk for vascular diseases.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the thrombolytic ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of vascular disease in the subject being treated. A therapeutically effective dose refers to that amount of the compound that results in plasma levels of the thrombolytic reagent which are sufficient to maintain the beneficial modulating effects. Determination of the effective amounts is well within the capability of those skilled in the art.

The effective dose may be determined using a variety of different assays. For example, assays may be utilized to determine levels of fibrinogen or fibrin split products in the blood of treated patients. In such instances, the effective dose of the thrombolytic reagent is that amount required to sustain normal levels of fibrinogen or fibrin split products in the body of the patient. Such doses may be determined by measuring for levels of fibrinogen (assay for measuring levels of fibrinogen is available from M.L.A.,Inc.) or fibrin split products (Thrombo-Wellco Test;MUREX, Inc.) in the blood of treated patients. A therapeutically effective dose refers to that amount of thrombolytic reagent sufficient to maintain normal circulating blood levels of about 2–4 mg/ml of fibrinogen, or, less than 10 mg/ml of fibrin split products.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician. It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response is not adequate (precluding toxicity).

In administering thrombolytic reagents to the patient, it is particularly important to monitor the patient for excessive bleeding or tendencies to bleed. A variety of different diagnostic tests, which are well known to those skilled in the art may be used to access the patients susceptibility to bleeding due to administration of the thrombolytic reagents. Such assays include a complete blood count (CBC), or a determination of prothrombin or partial prothrombin time.

The magnitude of a prophylactic dose of the t-PA in the management of vascular disease will vary with the patient to be treated and the route of administration. Again, it should be noted that the clinician or physician would know when to interrupt and/or adjust the treatment dose due to toxicity. The dose, and perhaps the dosage frequency, will also vary according to the age, body weight, and response of the individual patient.

In general, the total daily dose range of t-PA should be sufficient to achieve serum concentration levels ranging between 1 and 50 mgs. For example, between about 1 and 50 mgs of a daily parenteral dose may be administered, while most preferably a daily dose range should be between

about 10 and 30 mgs of a parenteral dose of t-PA. For smaller patients (less than 65 kg), a dose of 0.1–0.5 mg/kg may be administered daily. It is further recommended that infants, children, and patients over 65 years, and those with impaired renal, or hepatic function, initially receive low doses, and that they be titrated based on individual clinical response(s) and blood level(s). It may be necessary to use dosages outside these ranges in some cases as will be apparent to those of ordinary skill in the art.

THROMBOLYTIC DRUG DELIVERY SYSTEMS

A variety of drug delivery systems may be used to deliver the thrombolytic reagents, such as t-PA into the bloodstream of the patient. For example, the t-PA can be administered to a human patient in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses therapeutically effective to prevent a variety of vascular disorders. Suitable routes of administration may, for example, include transdermal, topical, oral, intranasal and the like. Dosage forms include but are not limited to aerosol dispersions, creams, patches and the like.

For purposes of clarity, the following discussion describes delivery systems for t-PA. However, the delivery systems are not so limited. It is understood that the delivery systems described below may also be utilized for delivery of other thrombolytic reagents such as urokinase and streptokinase. Techniques for formulation and administration of the thrombolytic reagents of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the t-PA into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Suitable routes of administration may, for example, include transdermal, topical, oral, intranasal and the like. Dosage forms include but are not limited to aerosol dispersions, creams, patches and the like.

The formulations of the present invention normally will consist of t-PA with a carrier, or diluted by a carrier. Some examples of the diluents or carriers which may be employed in the pharmaceutical compositions of the present invention are lactose, dextrose, sucrose, sorbitol, mannitol, propylene glycol, liquid paraffin, white soft paraffin, kaolin, microcrystalline cellulose, calcium silicate, silica polyvinylpyrrolidone, cetostearyl alcohol, starch, gum acacia, calcium phosphate, cocoa butter, oil of theobroma, arachis oil, alginates, tragacanth, gelatin, syrup B.P., methyl cellulose, polyoxyethylene sorbitan monolaurate, ethyl lactate and propylhydroxybenzoate, sorbitan trioleate, sorbitan sesquioleate and oleyl alcohol.

Because of the short shelf life of t-PA in solution, formulations of t-PA in aqueous solutions, gels, etc. are stored under refrigeration to preserve the activity of the t-PA. Lyophilized preparations of t-PA may be stored at room temperature and protected from excessive exposure to light without loss of activity.

A variety of different drug delivery systems may be used to deliver t-PA into the bloodstream of the patient. In one particular embodiment of the invention a dermal patch may be used for sustained delivery of t-PA into the body. These membrane systems are designed to deliver controlled doses of drugs through the skin into the bloodstream.

TRANSDERMAL DELIVERY SYSTEM

Transdermal delivery of t-PA can be designed so that the rate of delivery of the t-PA closely follows the rate of clearance of the t-PA from the patient's body, thus keeping constant levels of the t-PA in the blood, thereby reducing t-PA waste and overdosing. The use of such a drug delivery system also provides a comfortable, convenient non-invasive method for unattended delivery of t-PA over a prolonged time period.

The transdermal patches to be used in the practice of the invention may be obtained from any of a variety of commercial sources. Most patches consists of a reservoir of drug material located behind a rate controlling membrane. The patch is impregnated with the t-PA and placed on the skin of the patient which allows the drug to penetrate readily into the body. In the practice of the invention the transdermal patch will be periodically replaced as the t-PA becomes depleted.

The transdermal patch is prepared to contain a solution of t-PA. The t-PA is dispersed in the solution, suspension or gel in a dissolved or undissolved state. The drug reservoir of the patch containing a solution, suspension or gel of t-PA also includes permeation enhancers which increase the skin penetration of the t-PA. Such permeation enhancers include those described in U.S. Pat. No. 4,573,966, which is incorporated by reference herein. Permeation enhancers may include plasticizer type enhancers such as lower alky and alkoxy esters of pharmaceutically acceptable fatty acids, fatty acid esters, fatty alcohols and similar hydrophobic compounds that are capable of increasing the permeability of drugs to the skin. In addition, solvent type enhancers may be used to increase the delivery of drugs through the skin. Such enhancers generally refer to relatively hydrophilic compounds having molecular weights of less than 200. More preferably, solvent type enhancers have a molecular weight of less than 150. They are also generally greater than 2 wt % soluble in water, and are preferably greater than 10 wt % soluble in water. Typically, solvent type enhancers include pharmaceutically acceptable lower alkyl alcohol, aryl alcohol, or polyol, for example, ethanol, propanol, butanol, benzyl alcohol, glycerin, or propylene glycol. as well as diluents, such as water or other additives. The solution of t-PA may be formulated to include vascular permeability factors (VPFs), as described in U.S. Pat. No. 5,503,843, which cause a rapid and reversible increase in blood vessel permeability. Such VPF may be added to the t-PA solution to facilitate the uptake of t-PA into the blood vessels of the skin. In addition, gelling agents may be added to increase the viscosity of the solution as is described in U.S. Pat. No. 5,503,843. The t-PA may also include diluents, stabilizers, biocides, antioxidants, anti-irritants and the like.

Because of the instability of t-PA in solution, it is desirable to design transdermal patches that can be stored at room temperature. Such a dermal patch may be designed, for example, with two compartments separated by a breakable barrier; one compartment contains lyophilized t-PA and the other compartment contains a solution or carrier, such as those described above, into which the t-PA is dissolved. Prior to the use of the patch, the barrier is broken, mixing the contents of both compartments thereby forming a drug reservoir containing a solution of t-PA. Alternatively, a transdermal patch may be designed with a single breakable compartment containing lyophilized t-PA, enclosed within the liquid carrier. Prior to use of the patch, the single compartment barrier is broken releasing the lyophilized t-PA into the carrier solution. The patch is then placed in contact with the skin in such a way that the drug reservoir containing the t-PA solution is in contact with the skin.

INTRANASAL DELIVERY SYSTEM

In yet another embodiment of the invention, the t-PA may be administered intranasally. The large blood supply carried in the capillaries of the nose allow drugs to enter the bloodstream quickly. For administration by inhalation, t-PA are conveniently delivered in the form of an aerosol spray

presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

In addition, the inhalers may be formulated to include vascular permeability factors (VPFs) which cause an increase in blood vessel permeability thereby facilitating the uptake of t-PA into the blood vessels of the nose.

IMPLANTABLE DELIVERY SYSTEMS

In addition to the formulations described above, the t-PA may also be formulated as a slow release preparations that may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the t-PA may be formulated with suitable biocompatible matrix materials. The compounds may be delivered using a sustained-release system, such as slow release gel formations containing the t-PA. Various slow release gel formations have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the t-PA for prolonged periods of time.

ORAL FORMULATIONS

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic aced or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.

The t-PA is preferably formulated for oral administration with enteric coatings which protect the t-PA from enzymatic degradation in the stomach and promotes uptake by the intestinal tract. Such formulations are designed for slow release of t-PA through the intestinal wall and into the bloodstream of the patient. For example, the drug capsule containing t-PA may be coated with an enteric film which is sufficiently insoluble at a pH below 7 as to be capable of protecting the capsule and its contents from the digestive enzymes until the capsule reaches a region below the upper part of the intestine. Such film compositions include mixtures of anionic acrylic copolymers derived from at least on monomer selected from acrylic and methacrylic acids and methylacrylates. Such copolymers are commercially available under the trade name "Eudragit"(TM). Such enteric coatings are well known to those skilled in the art, and include those described in U.S. Pat. No. 4910021 and U.S. Pat. No. 5350741, each of which is incorporated by reference herein. Dyestuffs or pigments may also be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in a mixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in a conventional manner.

PARENTERAL FORMULATIONS

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulator agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

PACKAGING

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labelled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of patients at risk for development of vascular diseases, or alternatively treatment of patients suffering from vascular diseases such as cerebral vascular

thrombosis, pulmonary embolism, deep venous thrombos, acute myocardial infarction and fresh or aged arterial thrombi.

EXAMPLE

TRANSDERMAL ADMINISTRATION OF THROMBOLYTIC REAGENTS

The following example describes the administration of the thrombolytic reagent t-PA utilizing a transdermal patch delivery system. The use of transdermal patches for the delivery of drugs through the skin is well known. Methods for the use of transdermal patches for delivery of drugs is described, for example, in the following United States patents, U.S Ser. Nos. 5,498,417, 5,503,844 and 5,503,843, each of which is incorporated by reference herein.

The following example illustrates the invention. It is not intended to limit the scope of the invention.

The t-PA (Activase, supplied by GENENTECH, Inc.) to be used in this example is supplied in 50 mg vials. The vials should be reconstituted in either sterile water or a pharmaceutical composition compatible with use in a transdermal patch.

The transdermal patch is prepared to contain a solution of t-PA. The t-PA is dispersed in the solution, suspension or gel in a dissolved or undissolved state. The drug reservoir of the patch containing a solution, suspension or gel of t-PA also includes permeation enhancers which increase the skin penetration of the t-PA. Such permeation enhancers include those described in U.S. Pat. No. 4,573,966, which is incorporated by reference herein. Permeation enhancers may include plasticizer type enhancers such as lower alky and alkoxy esters of pharmaceutically acceptable fatty acids, fatty acid esters, fatty alcohols and similar hydrophobic compounds that are capable of increasing the permeability of drugs to the skin. In addition, solvent type enhancers may be used to increase the delivery of drugs through the skin. Such enhancers generally refer to relatively hydrophilic compounds having molecular weights of less than 200. More preferably, solvent type enhancers have a molecular weight of less than 150. They are also generally greater than 2 wt % soluble in water, and are preferably greater than 10 wt % soluble in water. Typically, solvent type enhancers include pharmaceutically acceptable lower alkyl alcohol, aryl alcohol, or polyol, for example, ethanol, propanol, butanol, benzyl alcohol, glycerin, or propylene glycol. as well as diluents, such as water or other additives. The solution of t-PA may be formulated to include vascular permeability factors (VPFs), as described in U.S. Pat. No. 5503843, which cause a rapid and reversible increase in blood vessel permeability. Such VPF may be added to the t-PA solution to facilitate the uptake of t-PA into the blood vessels of the skin.

The amount of t-PA contained in the patch is that amount necessary to deliver a daily dose of between 1–50 mg of t-PA. The treated patient's blood is monitored to determine the levels of circulating fibrinogen and/or fibrin split products. The amount of t-PA contained in the patch is adjusted so as to maintain blood levels of about 2–4 mg/ml of fibrinogen and 10 mg/ml of fibrin split products. In addition, the treated patient is monitored to prevent excessive bleeding which can result from treatment with thrombolytic reagents.

Once the transdermal patch has been prepared to contain an appropriate dose of t-PA, in a suitable solution, the patients skin is overlaid with the transdermal patch. The patch is placed in contact with the skin in such a way that the side of the patch containing the t-PA solution side is in contact with the patient's skin.

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the claims.

What is claimed:

1. A method for prevention of thrombotic vascular disease in a mammal, comprising the chronic administration to a patient in need thereof of an effective dose of a thrombolytic reagent to a mammal.

2. The method of claim **1** wherein the thrombolytic reagent is human tissue plasminogen activator.

3. The method of claim **1** wherein the thrombolytic reagent is streptokinase.

4. The method of claim **1** wherein the thrombolytic reagent is urokinase.

5. The method of claim **1** wherein the thrombolytic reagent is delivered in a transdermal patch.

6. The method of claim **5** wherein the thrombolytic reagent is selected from the group consisting of human tissue plasminogen activator, streptokinase and urokinase.

7. The method of claim **2** wherein the human tissue plasminogen activator is recombinant human tissue plasminogen activator.

8. The method of claim **1** wherein the thrombolytic reagent is delivered internasally.

9. The method of claim **8** wherein the thrombolytic reagent is selected from the group consisting of human tissue plasminogen activator, streptokinase and urokinase.

10. The method of claim **1** wherein the thrombolytic reagent is delivered topically in a topical cream.

11. The method of claim **10** wherein the thrombolytic reagent is selected from the group consisting of human tissue plasminogen activator, streptokinase and urokinase.

12. The method of claim **1** wherein the thrombolytic reagent is delivered orally.

13. The method of claim **12** wherein the thrombolytic reagent is selected from the group consisting of human tissue plasminogen activator, streptokinase and urokinase.

14. The method of claim **1** wherein the dose of the thrombolytic reagent is that dose sufficient to maintain circulating blood levels of 2–4 mg/ml of fibrinogen or less than 10 mg/ml of fibrin split products.

15. The method of claim **1** wherein the dose of the thrombolytic reagent is that dose sufficient to maintain circulating blood levels of less than 10 mg/ml of fibrin split products.

16. The method of claim **2** wherein the daily dose of t-PA is between 1–50 mg.

17. The method of claim **2** wherein the daily dose of t-PA is between 10–30 mg.

* * * * *

Exhibit B

AJH 2004; 17:823–827

Effect of Amlodipine–Atorvastatin Combination on Fibrinolysis in Hypertensive Hypercholesterolemic Patients With Insulin Resistance

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Background: The aim of this study was to evaluate the effect of the amlodipine–atorvastatin combination on plasma tissue plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1) activity in hypercholesterolemic, hypertensive patients with insulin resistance.

Methods: The study population included 45 patients, aged 41 to 70 years, with mild to moderate essential hypertension (diastolic blood pressure [BP] ≥95 and ≤105 mm Hg), hypercholesterolemia (total cholesterol >200 and <350 mg/dL), and insulin resistance (HOMA index >2.5) After a 4-week wash-out period, they were randomized to amlodipine (5 mg) or atorvastatin (20 mg) or their combination at the same oral dosage for 12 weeks in three cross-over periods each separated by a 4-week placebo period (3 by 3 latin square design). At the end of the placebo wash-out and of each treatment period, office BP, total cholesterol, PAI-1, and t-PA activity were evaluated.

Results: The amlodipine–atorvastatin combination, in addition to the expected hypocholesterolemic effect, produced: 1) a greater decrease in PAI-1 activity (−10.2 U/mL, $P < .01$ *v* placebo) and an even greater increase in t-PA activity (+0.26 U/mL, $P < .01$ *v* placebo) than amlodipine (−0.5 U/mL for PAI-1, P = not significant; +0.17 U/mL for t-PA, $P < .01$ *v* placebo) and atorvastatin alone (respectively, −9.9 U/mL, $P < .01$ *v* placebo and +0.08 U/mL, $P < .05$ *v* placebo); and 2) a greater systolic BP/diastolic BP mean reduction (−22/17 mm Hg, $P < .005$ *v* placebo) than amlodipine (−18/14 mm Hg, $P < .01$ *v* placebo) and atorvastatin alone (−2.8/3.8 mm Hg, $P < .05$ *v* placebo only for diastolic BP).

Conclusions: The positive effect on fibrinolytic balance and BP control observed suggests that in hypertensive, hypercholesterolemic patients with impaired fibrinolysis, the combination of amlodipine and atorvastatin could be the treatment of choice. Am J Hypertens 2004;17:823–827 © 2004 American Journal of Hypertension, Ltd.

Key Words: Amlodipine, atorvastatin, fibrinolysis, hypertension, hypercholesterolemia.

Population-based data have indicated that the two most common cardiovascular risk factors, hypertension and hypercholesterolemia, coexist in a large proportion of patients and their combination is associated with a rate of cardiovascular complications that greatly exceeds the separate contribution of any single risk factor.[1,2] In hypertension, a close relationship has also been demonstrated between disorders of lipid metabolism, insulin resistance, and impaired fibrinolysis, mainly expressed as increased plasminogen activator inhibitor type 1 (PAI-1) levels and depressed tissue plasminogen activator (t-PA) activity.[3–5] Endothelial dysfunction might be the pathogenetic link between these risk factors whose clustering greatly accelerates the atherogenic process and its clinical complications.[6] To achieve a reduction in both cardiovascular morbidity and mortality, current hypertension treatment guidelines stress the role of total risk factor management and state not only to lower blood pressure (BP) values but also to normalize high cholesterol and improve the global risk profile of hypertensive patients.[6]

The 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, commonly referred to as statins,

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are the most powerful agents available for the treatment of patients with hypercholesterolemia. Statins have demonstrated a capability to reduce the rate of cardiovascular events.[7–9] Data from the recent Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) support the view that statins protect hypertensive patients from end-organ damage, not only through cholesterol reduction but also through other pathways.[10] These include a direct modulation of the endothelial function, as well as an interaction with the fibrinolytic activity.[11,12] In this regard, evidence from in vitro studies indicate that statins positively affect the fibrinolytic system of cultured smooth muscle cells as well as endothelial cells.[13–15] Also some in vivo studies demonstrated that statins decreased PAI-1 plasma levels and increased t-PA activity.[16,17] Whether statin treatment may affect fibrinolytic activity has been poorly investigated in hypercholesterolemic, hypertensive patients pharmachologically treated for both risk factors.

Given this background, the present study was undertaken to evaluate the effect of the combination of the dihydropyridine calcium antagonist amlodipine and the HMG-CoA reductase inhibitor atorvastatin on plasma PAI-1 and t-PA activity in hypercholesterolemic, hypertensive patients with insulin resistance, a condition characterized by impaired fibrinolysis.[18]

Methods

The study population was selected according to the following inclusion criteria: outpatients of either sex, aged 56.3 ± 5.1 years, with mild to moderate essential hypertension (diastolic BP >90 and ≤105 mm Hg), total cholesterol (TC) >200 and <350 mg/dL, and insulin resistance, as defined by HOmeostasis Model Assessment (HOMA) Index >2.5. The HOMA Index (= glucose in millimoles per liter × insulin in microunits per milliliter/22.5) has been shown to correlate well with insulin resistance using clamp techniques.[19]

Patients with a diagnosis of diabetes, liver or kidney disease, cancer, major cardiovascular complication (myocardial infarction or unstable angina within 6 months, congestive heart failure), using corticosteroids or hormone replacement therapies, and having any other diseases with a poor prognosis were exluded from the study. Secondary forms of hypertension were excluded according to standard routine clinical and laboratory examination. The study protocol was approved by the local Ethical Committee and informed consent was obtained from each participant at the time of enrollment.

After an initial 4-week wash-out period, patients were randomly assigned to receive amlodipine (5 mg) or atorvastatin (20 mg) or their combination at the same oral dosage for 12 weeks in three cross-over periods each separated by a 4-week placebo wash-out period (3 by 3 latin square). At the end of the placebo wash-out and of each treatment period, office BP, TC, HDL cholesterol, LDL cholesterol, triglycerides, plasma PAI-1, and t-PA activity were evaluated. The BP measurements were obtained from each patient in the seated position using a standard mercury sphygmomanometer (Korotkoff I and V). Measurements were taken in the morning before daily drug intake (ie, 24 h after dosing) and after the subject had rested 10 min in a quiet room. Three successive BP readings were obtained at 1-min intervals and averaged. For evaluation of lipid and fibrinolytic parameters, blood was always drawn in the morning, between 8 and 9 AM, after a 15-min rest and after an overnight fast, to reduce interference by the diurnal variation of the PAI-1 and tPA.[20] The TC and TG were determined by the enzymatic method of the Chemetron Company (Frankfurt, Germany). The HDL cholesterol was determined by the enzymatic method of Roschlau[21] after LDL and very low-density lipoprotein (VLDL) precipitation with polyethylene glycol 6000 by the method of Viikari.[22] The LDL cholesterol was calculated by the formula of Friedewald et al.[23]

For fibrinolytic measurements, blood samples were collected in Biopool stabilyte tubes with citrate buffer, at pH 4.5, to ensure the stability of t-PA activity without affecting the assay of PAI-1 activity. Plasma was separated within 1 h by centrifugation for 20 min at 3000 *g* and stored at −70°C until assay. Plasma t-PA activity was determined with a parabolic rate assay based on fibrin stimulation of the t-PA-catalyzed conversion of Glu-plasminogen to plasmin, which subsequently cleaves the chromogenic substrate.[24] The t-PA activity was expressed in international units per milliliter by reference to the World Health Organization First International Standard for t-PA coded 86/670 from the National Institutes for Biological Standard and Control, Potters Bar, England. Plasma PAI-1 activity was determined with a two-stage, indirect enzymatic assay based on the addition of excess t-PA (40 UI) to the samples and measurement of the residual t-PA activity.[25] One unit of PAI-1 activity was defined as the amount of PAI-1 that inhibits 1 UI of international t-PA standard. The reagent kits for assay of t-PA and PAI-1 activities were purchased from Biopool AB, Umea, Sweden. The coefficients of variation for repeated measures of PAI-1 activity and t-PA activity in our laboratory were 5% and 8.5%, respectively.

Data are expressed as mean ± standard deviation. The statistical analysis was conducted by using SAS version 8 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) for the cross-over design (general linear model procedure) was used to analyze the results. Statistical significance was set at $P < .05$. To verify the basic of the crossover design,[26] the possibility of a carry-over or sequence effect was also investigated using the crossover ANOVA test.

Results

Forty-five patients, 22 men and 23 women, aged 41 to 70 years, were enrolled in the study and 41 patients completed it. Four patients dropped out, one because of side effects and three for lack of cooperation.

Table 1. Effect of each treatment on blood pressure, lipids, and fibrinolytic parameters

	Baseline	Atorvastatin 20 mg	Amlodipine 5 mg	Amlodipine + Atorvastatin
SBP (mm Hg)	158.3 ± 11.1	155.5 ± 11.2	140.3 ± 9.9†	136.1 ± 9.9‡
DBP (mm Hg)	96.1 ± 4.5	92.3 ± 4.4*	81.8 ± 4.1†	78.4 ± 4.2‡
TC (mg/dL)	264.3 ± 18.2	187.2 ± 14.3†	251.6 ± 16.9	180.5 ± 13.9†
HDL-C (mg/dL)	42.9 ± 5.1	46.2 ± 4.3*	43.1 ± 4.8	46.8 ± 4.1*
LDL-C (mg/dL)	193.5 ± 15.3	119.6 ± 11.6†	186.8 ± 15.2	111.6 ± 11.5†
TG (mg/dL)	139.3 ± 39	113.1 ± 33	134.2 ± 40	110.5 ± 34
PAI-1 (U/mL)	23.1 ± 11.3	13.2 ± 6.7*	22.6 ± 11.2	12.9 ± 6.8*
t-PA (U/mL)	0.51 ± 0.22	0.59 ± 0.14*	0.68 ± 0.20†	0.77 ± 0.25§

DBP = diastolic blood pressure; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PAI-1 = plasminogen activator inhibitor type 1; SBP = systolic blood pressure; TC = total cholesterol; TG = triglycerides; t-PA = tissue plasminogen activator.

Values expressed as mean ± standard deviation.

* $P < .05$ *v* placebo; † $P < .01$ *v* placebo; ‡ $P < .005$ *v* placebo; § $P < .05$ *v* amlodipine.

The main results of the study are reported in Table 1. As expected, amlodipine monotherapy was significantly effective in reducing both systolic BP (−18 mm Hg, −11.3%, $P < .01$ *v* placebo) and diastolic BP mean values (−14.3 mm Hg, −14.8%, $P < .01$ *v* placebo). Treatment with atorvastatin alone did not affect systolic BP values (−2.8 mm Hg, −1.7%, P = not significant), whereas it significantly reduced diastolic BP values (−3.8 mm Hg, −3.9%, $P < .05$ *v* placebo), although to a lesser extent compared with amlodipine. Interestingly, combination therapy with amlodipine plus atorvastatin produced a significantly greater reduction in both systolic BP (−22.2 mm Hg, −14%, $P < .005$ *v* placebo) and diastolic BP mean values (−17 mm Hg, −18.4%, $P < .005$ *v* placebo) than either drug alone.

Amlodipine monotherapy did not modify the lipid profile of the treated patients, whereas atorvastatin significantly reduced TC (−77.1 mg/dL, −29.1%, $P < .01$ *v* placebo) and LDL cholesterol levels (−73.9 mg/dL, −38.1%, $P < .01$ *v* placebo) and increased HDL cholesterol (+3.3 mg/dL, +7.6%, $P < .05$ *v* placebo), without affecting TG levels. Adding amlodipine did not significantly modify the lipid-lowering effect of atorvastatin.

Treatment with amlodipine alone did not affect plasma PAI-1 activity (−0.5 U/mL, −2.1%, P = not significant), whereas significantly increased t-PA activity (+0.17 u/mL, +33.3%, $P < .01$ *v* placebo). Atorvastatin monotherapy significantly decreased PAI-1 activity (−9.9 U/mL, −42.8%, $P < .01$ *v* placebo) and increased t-PA activity (+0.08 U/mL, +16.6%, $P < .05$ *v* placebo). The amlodipine–atorvastatin combination produced a significantly greater reduction in PAI-1 activity (−10.2 U/mL, −44.1%, $P < .01$ *v* placebo) and an even greater increase in t-PA activity (+0.26 U/mL, +50.9%, $P < .01$ *v* placebo and $P < .05$ *v* amlodipine) than either drug alone. No relationship was find between the changes in plasma PAI-1 and t-PA activity and the hypocholesterolemic effect or the BP lowering produced by the amlodipine–atorvastatin combination.

Discussion

The results of this study showed that in hypercholesterolemic, hypertensive patients with impaired fibrinolysis, the amlodipine–atorvastatin combination, beyond the expected hypocholesterolemic effect: 1) improved the fibrinolytic balance by decreasing the PAI-1 activity and particularly by increasing t-PA activity more than the single monotherapies; and 2) decreased both systolic and diastolic BP levels more than either drug alone.

The most original findings of our study were those regarding the effects of the amlodipine–atorvastatin combination on the fibrinolytic system. In agreement with some previous observations,[27] amlodipine monotherapy did not modify PAI-1 activity, whereas it significantly increased t-PA activity. Mechanisms for such an effect are unknown, although a direct action of amlodipine on vascular endothelium is likely to play an important role. Amlodipine has been suggested to improve endothelial function, mainly through an antioxidant action.[28,29] Because both PAI-1 and t-PA are synthetized in the vascular endothelium and endothelial dysfunction induces an imbalance in fibrinolysis,[30,31] improving endothelial function might reverse the fibrinolytic imbalance.

The results obtained with atorvastatin monotherapy (ie, a significant decrease in PAI-1 activity and an increase in t-PA activity) confirm the findings of some in vitro and in vivo studies.[13–17] Statins have been shown to reduce PAI-1 production in cultured human endothelial and smooth muscle cells and to increase t-PA production in human smooth muscle cells.[13] In addition, they increased fibrinolytic activity in tumor necrosis factor-α-activated human peritoneal mesothelial cells[15] and downregulated the synthesis of PAI-1 in cultured human monocytes.[14] Although the results of in vivo studies are more controversial, in some studies statins decreased plasma PAI-1 and increased t-PA activity.[14,15] The mechanism by which statins inhibit PAI-1 and increase t-PA expression appears

to be directly associated with geranylgeranylation of some cell proteins.[12,14,32,33]

Interestingly, in the present study the combination of amlodipine and atorvastatin improved the fibrinolytic balance more than the single monotherapy. In particular, a greater decrease in PAI-1 activity (−44%) and even a greater increase in t-PA activity (+51%) were observed. These results, which were independent from the changes in TC and BP levels induced by the amlodipine–atorvastatin combination, could be related to an additive effect of the two drugs at the endothelial level.

This study also demonstrated that the use of atorvastatin in addition to amlodipine in patients with hypertension and high cholesterol levels not only improved the lipid profile by reducing TC and LDL cholesterol and increasing HDL cholesterol levels, but also significantly improved BP control. This effect, which confirms previous observations of a positive interaction between statins and antihypertensive agents,[34,35] seems to be independent from the reduction in plasma cholesterol values and suggests the possibility of a positive synergistic interaction between atorvastatin and amlodipine. The rationale for such clinical synergism could involve a direct BP-lowering effect of statins, possibly related to an improvement of endothelium-mediated vasorelaxation and to reduced arterial stiffness and vasoconstriction.[15,36] Atorvastatin has been demonstrated to reduce BP in untreated hypertensive patients independently of its cholesterol-lowering effect[33,37] and also in the present study, atorvastatin monotherapy produced a significant reduction in diastolic BP values. Statins also seem capable of improving the sensitivity of the vessel wall to the vasodilating effect of antihypertensive drugs. Statins have been demonstrated to improve endothelium-dependent vascular function and cause a significant vasodilation.[38] This could result in a significant increase in the sensitivity of the vessel wall to the vasodilating action of amlodipine. Some retrospective analyses investigating the extent of the interaction between statins and different classes of antihypertensive drugs have shown that the effect on BP control was enhanced in patients who were given statins in combination with angiotensin-converting enzyme inhibitors and calcium channel blockers, whereas no significant interactions were observed with the use of β-blockers and diuretics.[3] The enhanced interaction between statins and drugs acting mainly at the level of the vascular wall (angiotensin-converting enzyme inhibitor and calcium channel blocker) support the hypothesis that treatment with statins may enhance the capability of some classes of drugs to reduce the peripheral tone and to improve the peripheral vasodilator capacity.[35]

From a clinical point of view, the additional BP reduction and the increased fibrinolytic activity observed by combining amlodipine and atorvastatin could significantly contribute to reducing the global cardiovascular risk in hypertensive, hypercholesterolemic patients and improve the overall preventive action of antihypertensive and lipid-lowering therapy. Such additional properties deserve further research.

In conclusion, the positive effect exerted by the amlodipine–atorvastatin combination on fibrinolytic balance and BP control, beyond its cholesterol-lowering effect, suggest that this combination could be the treatment of choice in hypertensive patients with hypercholesterolemia and impaired fibrinolysis.

References

1. Kannel WB: Risk stratification in hypertension: new insights from the Framingham Study. Am J Hypertens 2000;13(Suppl)3S–10S.
2. Borghi C: Interactions between hypercholesterolemia and hypertension: implications for therapy. Curr Opin Nephrol Hypertens 2002; 11:489–496.
3. Landin K, Tengborn L, Smith U: Elevated fibrinogen and plasminogen activator inhibitor (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. J Intern Med 1990; 227:273–278.
4. Jeng JR, Sheu WH, Jeng CY, Huang SH, Shieh SM: Impaired fibrinolysis and insulin resistance in patients with hypertension. Am J Hypertens 1996;9:484–490.
5. Tomijama H, Kimura Y, Mitsuhashi H, Kinouchi T, Yoshida H, Kushiro T, Doba N: Relationship between endothelial function and fibrinolysis in early hypertension. Hypertension 1998;31:321–327.
6. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ: The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. The JNC VII Report. JAMA 2003;289:2569–2572.
7. The Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) Study Group: Prevention of cardiovascular events and deaths with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. N Engl J Med 1998;339: 1349–1357.
8. Byington RP, Davis BR, Plehn JF, White HD, Baker J, Cobbe SM, Shepherd J: Reduction of stroke events with pravastatin: the Prospective Pravastatin Pooling (PPP) project. Circulation 2001;103: 387–392.
9. Schwartz GG, Olsson AG, Ezekowitz MD, Ganz P, Oliver MF, Waters D, Zeiher A, Chaitman BR, Leslie S, Stern T: Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes. The MIRACL Study: a randomized controlled trial. JAMA 2001;285:1711–1718.
10. Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes GT: Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower than average cholesterol concentrations in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA): a multicenter randomised controlled trial. Lancet 2003;361:1149–1158.
11. Takemoto M, Liao JK: Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Arterioscler Thromb Vasc Biol 2001;21:1712–1719.
12. Wolfrum S, Jensen K, Liao JK: Endothelium-dependent effects of statins. Arterioscler Thromb Vasc Biol 2003;23:729–736.
13. Wiesbauer F, Kaun C, Zorn G, Maurer G, Huber K, Wojta J: HMG CoA reductase inhibitors affect the fibrinolytic system in human vascular cells in vitro: a comparative study using different statins. Br J Pharmacol 2002;135:284–292.
14. Ishibashi T, Nagata K, Ohkawara H, Sakamoto T, Yokoyama K, Shindo J, Sugimoto K, Sakurada S, Takuwa Y, Teramoto T, Maruyama Y: Inhibition of Rho/Rho-kinase signaling downregulates plasminogen activator inhibitor-1 synthesis in cultured human monocytes. Biochim Biophys Acta 2002;1590:123–130.

15. Haslinger B, Kleemann R, Toet KH, Kooistra T: Simvastatin suppresses tissue factor expression and increases fibrinolytic activity in tumor necrosis factor-alpha-activated human peritoneal mesothelial cells. Kidney Int 2003;63:2065–2074.
16. Bevilacqua M, Bettica P, Milani M, Vago T, Rogolino A, Righini V, Santoli E, Norbiato G: Effect of fluvastatin on lipids and fibrinolysis in coronary artery disease. Am J Cardiol 1997;79:84–87.
17. Seljeflot I, Tonstad S, Hjermann I, Arnesen H: Improved fibrinolysis after 1-year treatment with HMG CoA reductase inhibitors in patients with coronary heart disease. Thromb Res 2002;105:285–290.
18. Vague Ph, Juhan-Vague I, Ailhoud MF, Badoer Ch, Viard M, Collen D: Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level and relative body weight in normal and obese subjects. Metabolism 1986;35:250–263.
19. Lansang MC, Williams GH, Carrol J: Correlation between the glucose clamp technique and the homeostasis model assessment in hypertension. Am J Hypertens 2001;14:51–53.
20. Kluft C, Jie AFH, Rijken DC, Verheijen JH: Daytime fluctuations in blood of tissue-type plasminogen activator (tPA) and its fasting inhibitor (PAI-1). Thromb Haemost 1989;59:329–332.
21. Roschlau P: Enzymatische bestimmung des gesamtcholesterin in serum. Z Klin Chem Klin Biochem 1974;12:403.
22. Viikari J: Precipitation of plasma lipoproteins by PEG-6000 and its evaluation with electrophoresis and ultracentrifugation. Scand J Clin Lab Invest 1976;36:265–271.
23. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
24. Wiman B, Mellbring G, Randy M: Plasminogen activator release during venous stasis and exercise as determined by a new specific assay. Clin Chim Acta 1983;127:279–288.
25. Chmielewska J, Randy M, Wiman B: Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. Thromb Res 1983;31: 427–436.
26. Senn SJ: Crossover Trials in Clinical Research. New York, John Wiley, 1993, pp 38–46.
27. Gleerup G, Winther K: Decreased fibrinolytic activity and increased platelet function in hypertension. Am J Hypertens 1991;4:168S–171S.
28. Ghiadoni L, Magagna A, Versari D, Kardosa I, Huang Y, Taddei S, Salvetti A: Different effect of antihypertensive drugs on artery endothelial function. Hypertension 2003;41:1281–1286.
29. Mason RPO: Atheroprotective effects of long-acting dihydropyridine-type calcium channel blockers: evidence from clinical trials and basic scientific research. Cerebrovasc Dis 2003;16(Suppl):11–17.
30. Lijnen HR, Collen D: Endothelium in hemostasis and thrombosis. Prog Cardiovasc Dis 1993;22(Suppl 4):S1–S14.
31. Rubanyi GM: The role of endothelium in cardiovascular homeostasis and diseases. J Cardiovasc Pharmacol 1993,22(Suppl 4):S1–S14.
32. Essig M, Nguyen G, Prie D, Escoubet B, Sraer JD, Friedlander G. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells: role of geranylgeranylation and Rho proteins. Circ Res 1998;83:683–690.
33. Swiatkowska M, Pawlowska Z, Szemraj J, Drzewoski J, Watala C, Cerniewski CS: Cerivastatin,a HMG-CoA reductase inhibitor, reduces plasminogen activator inhibitor-I (PAI-1) expression in endothelial cells by down-regulation of cellular signaling and inhibition of PAI-1 promoter activity. Jpn J Pharmacol 2002;90: 337–344.
34. Sposito AC, Mansur AP, Coelho OR, Nicolau JC, Ramires JA: Additional reduction in blood pressure after cholesterol lowering treatment by statins (lovastatin or pravastatin) in hypercholesterolemic patients using angiotensin converting enzyme inhibitors (enalapril or lisinopril). Am J Cardiol 1999;83:1497–1499.
35. Borghi C, Dormi A, Veronesi M, Immordino V, Ambrosioni E: Use of lipid lowering drugs and blood pressure control in patients with arterial hypertension. J Clin Hypertens 2002;4:277–285.
36. Ferrier KE, Muhlmann MH, Baguet JP, Cameron JD, Jennings GL, Dart AM, Kingwell BA: Intensive cholesterol reduction lowers blood pressure and large artery stiffness in isolated systolic hypertension. J Am Coll Cardiol 2002;39:1020–1025.
37. Glorioso N, Troffa C, Filigheddu F, Dettori F, Soro A, Parpaglia PP, Collatina S, Pahor M: Effect of the HMG-CoA reductase inhibitors on blood pressure in patients with essential hypertension and primary hypercholesterolemia. Hypertension 1999;34:1281–1286.
38. Kaesemyer WH, Caldwell RB, Huang J, Caldwell RW: Pravastatin sodium activates endothelial nitric oxide synthase independent of its cholesterol lowering actions. J Am Coll Cardiol 1999;33:234–241.

Exhibit C

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Ann C. Barry, Ph.D.
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September 14, 2005

Eugene Berman
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Dear Mr. Berman,

Your letter of August 5th to our Chairman, Hank McKinnell, regarding U.S. Patent No. 5,837,688 was referred to me since I reside in Pfizer's Licensing and Development Group and have responsibility for Cardiovascular Products.

The above mentioned patent covers the fibnrinolytic activity of numerous cardiovascular products and your proposal suggests that the patent may have relevance to Lipitor (atorvastatin calcium). Your proposal was therefore reviewed by a number of Pfizer individuals representing the cardiovascular scientific/medical discipline as well as from an IP perspective.

Lipitor has no indications that are dependent upon anti-thrombotic or fibrinolytic activity and there is no plan to pursue such an indication. The Pfizer team therefore concluded that the patent would have minimal value to Pfizer and that there was no interest in further discussing this licensing opportunity.

We thank you for offering this opportunity to Pfizer and we wish you well with the initiative.

Best regards,

Ann

cc: Alan Hesketh